

3. Gomez-de-Antonio D, Campo-Canaveral JL, Crowley S, Valdivia D, Cordoba M, Moradiellos J, et al. Clinical lung transplantation from uncontrolled non-heart-beating donors revisited. *J Heart Lung Transplant*. 2012;31:349-53.
4. Wigfield CH, Love RB. Donation after cardiac death lung transplantation outcomes. *Curr Opin Organ Transplant*. 2011;16:462-8.
5. Steen S, Sjoberg T, Pierre L, Liao Q, Eriksson L, Algotsson L. Transplantation of lungs from a non-heart-beating donor. *Lancet*. 2001;357:825-9.
6. Cypel M, Yeung JC, Hirayama S, Rubacha M, Fischer S, Anraku M, et al. Technique for prolonged normothermic ex vivo lung perfusion. *J Heart Lung Transplant*. 2008;27:1319-25.
7. Cypel M, Rubacha M, Yeung J, Hirayama S, Torbicki K, Madonik M, et al. Normothermic ex vivo perfusion prevents lung injury compared to extended cold preservation for transplantation. *Am J Transplant*. 2009;9:2262-9.
8. Cypel M, Yeung JC, Liu M, Anraku M, Chen F, Karolak W, et al. Normothermic ex vivo lung perfusion in clinical lung transplantation. *N Engl J Med*. 2011;364:1431-40.
9. LaPar DJ, Laubach VE, Emaminia A, Crosby IK, Hajzuz VA, Sharma AK, et al. Pretreatment strategy with adenosine A2A receptor agonist attenuates reperfusion injury in a preclinical porcine lung transplantation model. *J Thorac Cardiovasc Surg*. 2011;142:887-94.
10. Emaminia A, Lapar DJ, Zhao Y, Steidle JF, Harris DA, Laubach VE, et al. Adenosine A(2)A agonist improves lung function during ex vivo lung perfusion. *Ann Thorac Surg*. 2011;92:1840-6.
11. Gazoni LM, Laubach VE, Mulloy DP, Bellizzi A, Unger EB, Linden J, et al. Additive protection against lung ischemia-reperfusion injury by adenosine A2A receptor activation before procurement and during reperfusion. *J Thorac Cardiovasc Surg*. 2008;135:156-65.
12. Gazoni LM, Walters DM, Unger EB, Linden J, Kron IL, Laubach VE. Activation of A1, A2A, or A3 adenosine receptors attenuates lung ischemia-reperfusion injury. *J Thorac Cardiovasc Surg*. 2010;140:440-6.
13. Sharma AK, Laubach VE, Ramos SI, Zhao Y, Stukenborg G, Linden J, et al. Adenosine A2A receptor activation on CD4+ T lymphocytes and neutrophils attenuates lung ischemia-reperfusion injury. *J Thorac Cardiovasc Surg*. 2010;139:474-82.
14. Koroskenyi K, Duro E, Pallai A, Sarang Z, Kloor D, Ucker DS, et al. Involvement of adenosine A2A receptors in engulfment-dependent apoptotic cell suppression of inflammation. *J Immunol*. 2011;186:7144-55.
15. Linden J. Molecular approach to adenosine receptors: receptor-mediated mechanisms of tissue protection. *Annu Rev Pharmacol Toxicol*. 2001;41:775-87.
16. Lisle TC, Gazoni LM, Fernandez LG, Sharma AK, Bellizzi AM, Schifflett GD, et al. Inflammatory lung injury after cardiopulmonary bypass is attenuated by adenosine A(2A) receptor activation. *J Thorac Cardiovasc Surg*. 2008;136:1280-8.
17. Snell GI, Rabinov M, Griffiths A, Williams T, Ugoni A, Salamonsson R, et al. Pulmonary allograft ischemic time: an important predictor of survival after lung transplantation. *J Heart Lung Transplant*. 1996;15:160-8.
18. Thabut G, Mal H, Cerrina J, Darteville P, Dromer C, Velly JF, et al. Graft ischemic time and outcome of lung transplantation: a multicenter analysis. *Am J Respir Crit Care Med*. 2005;171:786-91.
19. Ingemansson R, Eyjolfsson A, Mared L, Pierre L, Algotsson L, Ekmehag B, et al. Clinical transplantation of initially rejected donor lungs after reconditioning ex vivo. *Ann Thorac Surg*. 2009;87:255-60.
20. Weng Y, Sun S. Therapeutic hypothermia after cardiac arrest in adults: mechanism of neuroprotection, phases of hypothermia, and methods of cooling. *Crit Care Clin*. 2012;28:231-43.
21. Lampe JW, Becker LB. State of the art in therapeutic hypothermia. *Annu Rev Med*. 2011;62:79-93.
22. Globus MY, Busto R, Lin B, Schnipper H, Ginsberg MD. Detection of free radical activity during transient global ischemia and recirculation: effects of intracerebral brain temperature modulation. *J Neurochem*. 1995;65:1250-6.
23. Yang D, Guo S, Zhang T, Li H. Hypothermia attenuates ischemia/reperfusion-induced endothelial cell apoptosis via alterations in apoptotic pathways and JNK signaling. *FEBS Lett*. 2009;583:2500-6.
24. Akriotis V, Biggar W. The effects of hypothermia on neutrophil function in vitro. *J Leukoc Biol*. 1985;37:51-61.
25. Dempsey RJ, Combs DJ, Maley ME, Cowen DE, Roy MW, Donaldson DL. Moderate hypothermia reduces postischemic edema development and leukotriene production. *Neurosurgery*. 1987;21:177-81.
26. Fischer S, Renz D, Wiesnet M, Schaper W, Karliczek GF. Hypothermia abolishes hypoxia-induced hyperpermeability in brain microvessel endothelial cells. *Brain Res Mol Brain Res*. 1999;74:135-44.

Discussion

Dr David P. Mason (Cleveland, Ohio). Dr Mulloy, congratulations on an impressive experiment and presentation. It is obvious you put a lot of thought into the study design, and I am certain the execution took a great amount of patience, as well as technical expertise. I do not have any criticisms, although I do have 3 questions.

My first question. You designed a model of Maastricht category I non-heart-beating donation with a 1-hour period of no-touch warm ischemic time. Most lung transplantation performed throughout the world and all of it in the United States is with Maastricht category III donors, which have a much shorter no-touch time. Could you explain why you chose this model when it seems that even category III donors are underutilized?

My second question pertains to the use of systemic heparin. In your model, you gave no systemic heparin to the donor, and at harvest you noted a large amount of clot that you made great efforts to remove. I wonder what role microscopic thrombosis played in your graft function? Did you note any microscopic thrombi on histopathologic examination after animal death?

My last question. Your finding that immediate EVLP performed worse than delayed EVLP is surprising. You suggest that this is explained by the protective effect of hypothermia that is better achieved in the delayed EVLP during the 4-hour waiting period on ice. After flushing with cold Perfadex, did the immediate EVLP group receive any time on ice?

Thank you very much, and an excellent presentation and study.

Dr Mulloy. Thank you Dr Mason, for your kind comments and excellent questions. I will answer them in the order that you asked.

The first question regarding why we chose Maastricht category I versus III. Based on the experience from the Toronto group, we know that Maastricht category III lungs can be successfully assessed and rehabilitated for subsequent transplantation using EVLP. In their human clinical trial published last year, the Toronto group showed that recipients of category III lungs that were assessed by EVLP had equivalent clinical outcomes to standard lung transplant recipients. Thus, we really designed this study as a "proof of concept" study to demonstrate that uncontrolled Maastricht category I donors could also be rehabilitated for successful transplantation. That is why we chose a relatively longer ischemic time of 60 minutes, basically with the idea that if we could recondition these lungs with extended warm ischemic times for successful transplantation, we would prove that a significant proportion of uncontrolled non-heart-beating donor lungs could be reconditioned.

Second, although the Maastricht category III donors are surely underused, just from a pure numbers standpoint, the use of category I donors would go much further toward solving the donor organ shortage. Several hundred thousand people each year die of cardiac arrest, and if we could use even 1% of those lungs, it would go a long way toward solving the donor organ shortage.

Regarding the second question about the lack of heparin dosing and the presence of microscopic thrombi, it is a great question, because thrombosis definitely did play a role in our study. At lung explantation, we did note clot both in the left atrium and in the pulmonary veins, and we made all efforts to remove that clot. However, clearly we were not able to remove all the clot, and I am sure there was additional clot in the capillaries and smaller vessels that we were unable to even visualize. I asked our

histopathologist about the presence of thrombi, and he did not notice any mature fibrin thrombi in any of our samples. That being said, the EVLP-treated lungs had heparin added to the acellular perfusate, and also our recipient pigs were heparinized during the 4-hour reperfusion period. Thus, I think the heparin during the EVLP perfusion and recipient reperfusion likely dissolved any existing thrombi, and I suspect that if we looked at the lung histopathologic features immediately at explantation, we would see extensive microscopic thrombi.

In fact, probably 1 of the great benefits of EVLP is that during the acellular perfusion period you can treat with heparin to dissolve any existing clot in these uncontrolled donors.

Finally, the last question about whether the immediate group received any hypothermia, the short answer to that question is yes. After lung explantation, all lungs were taken to the back table and flushed retrograde with additional Perfadex. For the immediate EVLP group, we then sewed in the EVLP cannulas as quickly as possible, but that did take an average of 22 minutes. During that period, the lungs were immersed in cold Perfadex; however, for exposure reasons, they were not completely immersed. Thus, it was really 22 minutes of partial hypothermia. In this study, obviously, those 22 minutes of partial hypothermia were not adequate. Were the full 4 hours of hypothermia used in the D-EVLP group before EVLP necessary? Probably not. It is probably some period in the middle that is needed to achieve the observed beneficial hypothermic effect.

Dr Michael J. Weyant (*Aurora, Colo*). I thought your presentation was excellent and your project is very interesting. I had a couple of questions, and I realize that you will probably touch on these in later papers. Regarding the adenosine agonist treatment. I did not notice whether you had given any in your flush in your control group and whether you think that this plays a role in the successful outcome of the long-term EVLP group, and second, whether you did any analysis looking at whether the receptors were downregulated or not in your specimens. I am sure that you are planning on doing these. I am just curious to know.

Dr Mulloy. Thank you for that question. I am sure a lot of you know that our group has a long history of investigation into the use of adenosine agonists in lung ischemia–reperfusion injury.

We did add the adenosine 2A receptor agonist to the flush in all groups, and both of the EVLP groups received adenosine 2A agonist dosing during EVLP. Therefore, we did not have an untreated control group that would allow us to make specific statements about the role of the adenosine-2A agonist in this study. We recently published a study showing that the adenosine 2A agonist prevented the ischemia–reperfusion injury related to lung transplantation. We have also published our experience using a selective adenosine 2A agonist during EVLP in a nontransplant porcine model and demonstrated lung protection during EVLP with adenosine 2A receptor agonist. So again, with the present study designed mainly as a proof-of-concept study, we opted to add the

adenosine 2A agonist to all groups. Now that we have proved the concept, we plan to perform additional studies in which we will have appropriate controls to determine exactly what the role of the adenosine 2A agonist is in this model. Finally, we did not check receptor upregulation or downregulation in this study but plan to do so in future studies.

Dr Todd Rosengart (*Stony Brook, NY*). Congratulations on a great study, a great circumstance in which an unexpected result raises more questions than you anticipated. I think the obvious question is, have your results begat the additional experimental group of immediate cold reperfusion with or without agonists? Is this something you have contemplated or done already, and if not, what would you expect to find with that?

Dr Mulloy. We have not discussed performing cold reperfusion. I think 1 of the lessons learned in this study is that we obtained excellent lung function from the delayed EVLP group. Thus, perhaps with the additional use of EVLP, the standard cold static preservation period is not as damaging as is generally thought and could probably be extended a little bit longer in many circumstances. In my mind, the future translation of these results would be to transport donor lungs on ice, then place them on normothermic EVLP at certain centers that specialize in lung assessment and rehabilitation, and then send them back out on ice to individual hospitals for subsequent transplantation. However, regarding the role of cold perfusion, we have not discussed that because we believe that hypothermia is important for suppression of the inflammatory response but does not allow for true rehabilitation or appropriate functional assessment.

Dr Fabio De Robertis (*Harefield, United Kingdom*). Thank you. I enjoyed your presentation very much. Congratulations for your study.

That you did not administer systemic heparin is actually matching what happens in the clinical setting in the United Kingdom. We cannot heparinize the donor who experienced cardiac death. In the clinical setting, we use category III or IV donors. But did you add any heparin in the flush? Did you add anything else in the flush? Did you use prostaglandin? The second question is: did you do any retrograde flush, as well as antegrade flush? Did you use anything else in the flush? Did you use prostaglandin? How did you do that?

My third question is: of the lungs that did not improve in function, did you x-ray them, and if you did, what were the findings?

Dr Mulloy. I will answer your last question first. We did not x-ray the lungs because we do not at this time have x-ray capabilities in our animal operating theater.

Regarding the use of heparin, we did add 10,000 IU of heparin to the Perfadex flush and also performed a retrograde flush. We also infused prostaglandin E₁ into the pulmonary artery immediately before starting the flush. However, even with those maneuvers, there was still clot present. When we placed the lungs on EVLP, we would see clots continue to come out of the lungs for the first 15 to 30 minutes.